EXPERIMENTS ON LIGIA IN BERMUDA

VII. Further Effects of Sodium, Ammonium and Magnesium

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This paper is a continuation of a series dealing with the ecology and physiology of the littoral isopod *Ligia baudiniana*. In spite of its terrestrial life this interesting transitional form is morphologically equipped for life in the sea. The gills are well developed and their beating in sea water has temperature characteristics typical for respiratory processes (Barnes, 1936). The experiments were designed to determine how *Ligia* survives in air, what factors restrict it to the littoral zone and how long the animal survives in modifications of sea water. A brief summary of previous results will serve to indicate the significance of the new data presented below. Without entering the sea the *Ligia* draws water to the gills by capillarity (Barnes, 1938) but the young are released under water (Barnes, 1932). *Ligia* orients to the shore by negative phototropism in the sea and by positive geotropism on land (Barnes, 1932, 1935). Under experimental conditions *Ligia* survives about two days in natural sea water and in 25 per cent sea water with added calcium (Barnes, 1938). No artificial sea water more favorable than natural sea water has been found (Barnes, 1936). The toxicity series for the common cations in sea water is typical of other Crustacea (Barnes, 1932). The isopod shows a marked negative reaction to filter paper moistened with sea water (compared with distilled water). It was shown that this is probably an effect of sodium (Barnes, 1939). In brief, these studies aim to describe some of the factors regulating the life of a marine form which has recently invaded the intertidal zone.

Reactions to Filter Paper Moistened with Salt Solutions

To determine which ion in sea water repels *Ligia* from filter paper moistened with this medium, it was necessary to test the common ions of sea water separately. Previous experiments (Barnes, 1939) showed that Ca has no repellent action; and the concentration of K in sea water is below the threshold for the negative reaction. It remained to test Mg, the commonest ion after Na. As in previous experiments a filter paper 25 cm. in diameter was cut in two and each half saturated with a different medium. As will be seen in Table I, an equal distribution of isopods occurred between $MgCl_2$ and distilled water papers. Since this shows that magnesium, like calcium and potassium, does not produce negative reactions, sodium remains the only ion which can be responsible

TABLE I

Reaction of *Ligia* to filter paper saturated with salt solutions.

Treatment of each half of paper	Total number of isopods on each half	Number of experiments	Ratio
Normal adults			
5/8 M NaCl *vs.* sea water	32 : 205	4	1 : 6.40
5/8 M NaCl *vs.* 3.5/8 M $CaCl_2$	224 : 350	13	1 : 1.56
3.7/8 M $MgCl_2$ *vs.* distilled water	201 : 188	7	1 : 0.93
5/8 M NH_4Cl *vs.* distilled water	6 : 128	3	1 : 21.33
75% 5/8 M NH_4Cl *vs.* distilled water	1 : 54	1	1 : 54
50% 5/8 M NH_4Cl *vs.* distilled water	0 : 70	1	0 : 70
40% 5/8 M NH_4Cl *vs.* distilled water	5 : 48	1	1 : 9.60
33% 5/8 M NH_4Cl *vs.* distilled water	42 : 148	4	1 : 3.52
10% 5/8 M NH_4Cl *vs.* distilled water	19 : 132	3	1 : 7.00
5% 5/8 M NH_4Cl *vs.* distilled water	42 : 145	4	1 : 3.45
2% 5/8 M NH_4Cl *vs.* distilled water	103 : 92	4	1 : 0.89
Adults after 4 days in sea water			
Sea water *vs.* distilled water	35 : 34	1	1 : 1
Adults with antennae removed			
Sea water *vs.* distilled water	163 : 207	8	1 : 1.27
5/8 M NaCl *vs.* distilled water	52 : 127	4	1 : 2.44
Adults with spines and 7th leg removed			
Sea water *vs.* distilled water	26 : 65	2	1 : 2.50
Young just released from brood pouch			
Sea water *vs.* distilled water	102 : 192	4	1 : 1.88
5/8 M NaCl *vs.* distilled water	19 : 44	1	1 : 2.31

for the negative reaction to sea water. Having established that the sodium in sea water repels *Ligia*, the next step was to determine the distribution of specimens between sea water and NaCl paper. It is interesting to note that the NaCl alone proved to be six times as repellent as sea water.

Former experiments (Barnes, 1939) showed that the most marked negative reaction occurs to KCl paper, the magnitude of the response being a rectilinear function of the concentration. It is known that NH_4 has physiological effects similar to K on *Ligia* (Barnes, 1935) and other invertebrates (Wells, 1928). Accordingly, the reaction to NH_4Cl paper was tested (Table I). The repellent action of NH_4 proved to be unusually strong.

It was suggested (Barnes, 1938) that stimulation of the legs by the KCl and NaCl papers forced the isopods over on the distilled water paper. This hypothesis was supported by tests showing the tendency to collect on $CaCl_2$ paper as opposed to NaCl. The former results were based on the repeated orientation of the same four isopods. Accordingly, this experiment was repeated on a large scale (Table I). The preference for $CaCl_2$ was again evident but the ratio between Ca and Na paper was lower, owing to the greater number of specimens tested.

TABLE II

Survival of *Ligia* in modified sea water.

Parts of salt solution in 100 cc. of modified sea water	Average survival	Maximum survival	Number of specimens
	hours	*hours*	
15 per cent 3.7/8 M $MgCl_2$......	80.3±8.5	144	20
33 per cent 3.7/8 M $MgCl_2$......	27.0±2.9	63	19
50 per cent 3.7/8 M $MgCl_2$......	12.9±0.6	22	18
80 per cent 3.7/8 M $MgCl_2$......	5.9±0.4	7	10
5 per cent 5/8 M NH_4Cl........	9.3±0.6	24	20
10 per cent 5/8 M NH_4Cl.......	4.1±0.4	7	10
15 per cent 5/8 M NH_4Cl.......	2.3±0.1	3	10
20 per cent 5/8 M NH_4Cl.......	1.6±0.08	2.5	20

Previous immersion in distilled water is known to prevent the negative reaction to sea water paper compared with distilled water paper (Barnes, 1939) and the present experiments (Table I) indicate that four days immersion in sea water also destroys this reaction. The purpose of these tests was to determine if the salt requirements of the animals conditioned their reactions to filter paper containing the various solutions employed.

It seemed desirable to find out what part of the animal was affected by the salt solution on the substratum. There are three possibilities: the antennae, the legs, and the uropodal spines. It will be seen in Table I that the removal of the antennae did not prevent the negative reaction to sea water. The removal of the uropods with spines together with the seventh leg eliminated the flushing mechanism to the gills. It will be recalled that water rises between the sixth and seventh legs, passes over the gills and drains down between the uropods and spines. The results presented in Table I indicate that this flushing circuit is not essential for the sea water effect.

It was reported in earlier papers that the young are released under water. Hence it became of interest to test the reactions of young isopods immediately after escape from the brood pouch. From the results (Table I) it is clear that *Ligia* begins its life history with an innate negative reaction to NaCl and to sea water. The young were tested in groups of ten on small filter papers (9 cm. in diameter).

Survival in Sea Water with Added $MgCl_2$ or NH_4Cl

The toxicity series for the common ions occurring in sea water was established as a possible means of detecting any special ionic effect on

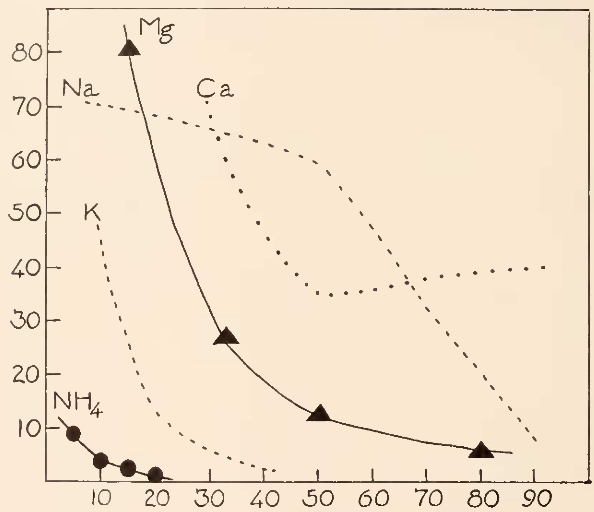

FIG. 1. Survival times of isopods in sea water in which the concentration of one ion is increased. Ordinates: average survival in hours. Abscissae: concentration of 5/8 M KCl, NaCl, and NH_4Cl and of 3.5/8 M $CaCl_2$ and 3.7/8 M $MgCl_2$ in cc. per 100 cc. of modified sea water. Triangles: $MgCl_2$; solid circles: NH_4Cl. The curves for KCl, NaCl, and $CaCl_2$ previously determined (Barnes, 1939) are indicated as dotted lines for comparison with the new data on $MgCl_2$ and NH_4Cl. Note the severe toxicity of NH_4Cl.

Ligia which might help to explain the limited survival in sea water. However, the series for single salts is the normal one for Crustacea. The next step was to determine the series for samples of sea water in which the concentration of one ion was progressively increased. This afforded more natural conditions. The graph published in the preceding paper did not include $MgCl_2$ and NH_4Cl. The present experiments (Table II and Fig. 1) were performed to complete this series. It is apparent from the data that these two ions, when tested in sea water, show the same relative toxicity as the single salts.

Effect of Oxygen in Diluted Sea Water

Earlier experiments indicated a limited survival of only about eighteen hours in 50 per cent sea water, but it was noted that the frequency of the gill beat increased. This suggested that continuous oxygenation of the diluted sea water might increase the longevity. Table III indicates that the survival in 50 per cent sea water is increased threefold by bubbling oxygen through the solution. The isopods were tested singly in finger bowls containing 100 cc. of solution. The level of the solution was marked on the bowl and distilled water was added from time to time to compensate for evaporation. Oxygenation

TABLE III

Survival of *Ligia* in natural sea water and dilutions.

Conditions	Average survival	Maximum survival	Number of specimens
	hours	*hours*	
Young just released from brood pouch. Each in 20 cc. of sea water	121.8± 4.9	148	14
Adults. Each in 1000 cc. of sea water	85.1±16.6	195	10
Adults. Each in 100 cc. of oxygenated 25% sea water	11.6± 0.4	16	16
Adults. Each in 100 cc. of oxygenated 50% sea water	60.2±11.5	96	10

had little effect on the survival in 25 per cent sea water. This was similar to previous results with single salts (Barnes, 1939).

Survival of Young Ligia in Natural Sea Water

It was reported (Barnes, 1935) that only immature specimens have been found in the sea and that the very young isopods are too small to migrate landward with the large specimens when the tide rises. These observations are now correlated with the immersion tests reported in Table III showing that recently released young survive for an average of 122 hours in sea water compared with 85 hours for adult controls tested at the same time. The mature specimens which served as controls were each immersed in 1 liter of sea water to compensate for the small size of the young (2.5 mm.), each in 20 cc. of sea water.

Discussion

The significance of the experimental results is indicated in the preceding sections of this paper to relate the new data to previous reports. The evidence is now fairly complete that sodium is responsible for the negative reaction to filter paper containing sea water. This result was rather unexpected as NaCl is the least toxic of the common cations for *Ligia*. The reactions of the isopods to substrata containing salts is undoubtedly modified by their previous history. Immersion in distilled water or sea water abolishes the preference for distilled water paper compared with sea water paper. Bateman (1933) showed that salts are concentrated by evaporation in specimens in air and as Miller (1938) points out, lost water must be replaced. This may be a factor in the collection of animals on the distilled water side of the filter paper. However, the actual flushing of the gills is not necessary for this reaction as the ablation experiments show. Nor are the antennae essential although the aversion for sea water is not so marked in specimens with antennae removed. This is seen in an experiment not listed in Table I. Isopods with antennae removed and others with uropods and seventh leg removed were allowed to orient in the same dish on filter paper containing sea water on one half and distilled water on the other. The ratios for sea water vs. distilled water were: for antennaless specimens, 21:45; for spineless specimens, 8:60.

Both the graphs for sea water containing additional Mg and NH_4 indicate severe toxic action not unlike the effect of K. The anaesthetizing action of $MgCl_2$ was evident at a concentration of ⅛ M in sea water. These modifications of sea water in which the concentration of one ion is increased are of speculative interest in connection with the geo-chemistry of the sea. Thus the same amount of Na and K have been supplied to the sea from primary rocks, i.e. 16.8 and 15.0 grams per kg. of water, but at present these quantities are 10.7 and 0.37 grams per kilo. The low K content of the sea is ascribed to the very great adsorption of its ions on the finely divided hydrolysate sediments (cf. Goldschmidt, 1937).

The prolonged survival of *Ligia* in oxygenated 50 per cent sea water might be expected from the increased frequency of gill beat in diluted sea water. The increased respiration probably supplies the additional energy expended in resisting osmotic disturbances in hypotonic media (for references, cf. Barnes, 1934).

It is now clear that *Ligia* begins its life history in the sea. Of hundreds of " births " observed only one or two occurred out of sea water or solutions. Moreover, the young survive immersion longer than adults, although the aversion for sea water and NaCl on filter paper is evident from the moment they are released from the brood pouch.

Summary

1. Filter paper moistened with $\frac{5}{8}$ M NaCl is approximately six times as repellent to *Ligia* as sea water paper.

2. An equal distribution of isopods occurs between filter papers containing 3.7/8 M $MgCl_2$ and distilled water.

3. Of all ions tested, the most pronounced aversion is shown for NH_4Cl paper (in concentrations of $\frac{1}{30}$ M and above).

4. The tendency of *Ligia* to collect on a filter paper containing distilled water when the other paper contains sea water is not prevented by the removal of the antennae or by removal of the mechanism for flushing the gills (seventh legs and uropods).

5. Young isopods immediately after release from the brood pouch have a marked aversion for filter paper containing $\frac{5}{8}$ M NaCl or sea water. Their tolerance for immersion in sea water is greater than that of adults.

6. The distribution of *Ligia* on filter paper containing distilled water, sea water, or other salt solutions is probably determined by two factors: (*a*) the salt requirements of the organism and (*b*) the stimulating effect of salt on the leg movements.

7. The survival of *Ligia* in 50 per cent sea water is prolonged by oxygenation.

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